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Anti-HA High Affinity

Rat monoclonal antibody (clone 3F10)

Stabilized

Cat. No. 11 867 423 001 50 µg
Cat. No. 11 867 431 001 500 µg

Version December 2005

Store at +2 to +8°C

Product overview

Antibody type	Clone 3F10, rat IgG_1 monoclonal antibody
Formulation	Lyophilizate
Specificity	Anti-HA High Affinity (3F10) recognizes the HA peptide sequence [YPYDVPDYA] derived from the influenza hemagglutinin protein (1). The antibody recognizes its antigenic determinant even when the HA peptide epitope is introduced into unrelated recombinant proteins by a technique known as "epitope tagging".
Preparation of the antibody	Anti-HA High Affinity was obtained by immunisation of rats with a synthetic peptide (residues 76-111 of X47 hemaglutinin 1) coupled to keywhole limpet hemocyanin (KLH). Spleen cells were isolated and fused with P3-X63-Ag8.653 myeloma cells as previously described (17). Hybridoma supernatants were screened for binding to the immunogen and for specific binding to HA-epitope-tagged fusion proteins. Hybridomas secreting monoclonal antibodies specific for the HA-epitope were isolated and cloned by limiting dilution. Anti-HA High Affinity antibody was purified from bioreactor supernatants and lyophilized in the presence of proteinous stabilizers.
Storage/ stability	The lyophilized antibody is stable at +2 to +8°C until the expiration date printed on the label. The reconstituted antibody solution is stable for 3 months at +2 to +8°C. Alternatively, it can be stored in aliquots at −15 to −25°C. ***Note***: Repeated freezing and thawing should be avoided.
Reconstitution and storage	Dissolving the lyophilizate in 0.5 ml (50 µg package size) and 2.5 ml (500 µg package size) double dist. water results in a concentration of 100 µg IgG/ml and 200 µg IgG/ml respectively. Allow the antibody to stand and rehydrate for 10 min prior to use.
Application	Anti-HA High Affinity is used for the detection of native and recombinant "epitope tagged" HA proteins for western and dot blots, immunocytochemistry, immunoprecipitation, and ELISAs. The high-affinity antibody requires lower working concentrations (50 – 200 ng/ml), which results in lower crossreactivity (*i.e.*, western blots), and which makes it well-suited for immunoprecipitation. Since Anti-HA High Affinity is a rat monoclonal, it is possible to use it in conjunction with murine monoclonals for double labeling (see also ref. 20
Quality	Function test: Western blot

Background information	The Anti-HA High Affinity antibody (clone 3F10) recognizes the same epitope as clone 12CA5, which was originally used to study how the immune system recognizes the influenza hemagglutinin protein, a surface glycoprotein required for infectivity of the human virus (1). However, the principal use of the Anti-HA antibody is the detection and purification of proteins whose encoding DNA sequences have been fused to the HA epitope sequence by recombinant techniques, that is epitope tagging (2,3). The ability to prepare such epitope tagged proteins and locate them with the Anti-HA antibody in subsequent experiments has enabled researchers to determine (1–17): • The size, cellular localization, and abundance of proteins produced by newly discovered genes • Post-translational modifications of proteins • The movement of proteins within cell membranes • The identity of proteins within functional protein complexes • The function of proteins that are unstable, difficult to purify, or share epitopes with a number of other proteins However, cross-reacting bands have been reported in certain Western blot experiments using Anti-HA (12CA5) (18). Anti-HA High Affinity is a monoclonal antibody whose high affinity and low working concentration result in less cross reactivity when compared with other antibodies to the HA-epitope (19). Additionaly, since Anti-HA High Affinity is a rat monoclonal, it is possible to use it in conjunction with murine monoclonals for double labeling.

Western blotting

Before you begin	Directly conjugated secondary antibodies (*e.g.*, anti-rat peroxidase conjugate) may be used successfully with Anti-HA High Affinity. However, with certain sample material (*e.g.*, mammalian and yeast extracts) nonspecific reactivity has been observed which is due to the anti-rat secondary and the total protein loading (>10 µg). This is eliminated by using the indirect anti-rat biotin/streptavidin system and minimizing the loading protein concentration
Additional reagents required	• PVDF Western Blotting Membranes* • TBS containing 1% BSA* casein or Blocking Reagent* • TBS containing 0.1% Tween® 20 (TBST) • Streptavidin-POD* • BM Chemiluminescence Blotting Substrate (POD)*

Protocol

Please refer to the following table.

Step	Action
1	After electrophoresis and transfer of the proteins to a membrane (*e.g.*, PVDF Western Blotting Membranes*) block the membrane with TBS containing 1% BSA*, casein or Blocking Reagent*. ***Note***: Gel loading of less than 10 µg total protein is recommended.
2	Incubate the blot with 50 – 200 ng/ml Anti-HA High Affinity in TBS containing 1% BSA or Blocking Reagent for 1 h at +15 to +25°C.
3	Wash 3× 5 min each, with TBS containing 0.1% Tween 20 (TBST).
4	Incubate the blot with Anti-Rat-Ig-Biotin (20 ng/ml) in TBS containing 1% BSA or Blocking Reagent for 30 min +15 to +25°C.
5	Wash 3×, 5 min each, with TBST.
6	Incubate the blot with Streptavidin-POD (5–15 mU/ml) in TBS containing 1% BSA or Blocking Reagent for 30 min at +15 to +25°C.
7	Detect bound antibody for high sensitive detection with a chemiluminescence substrate [*e.g.*, BM Chemiluminescence Blotting Substrate (POD)*].

Typical result

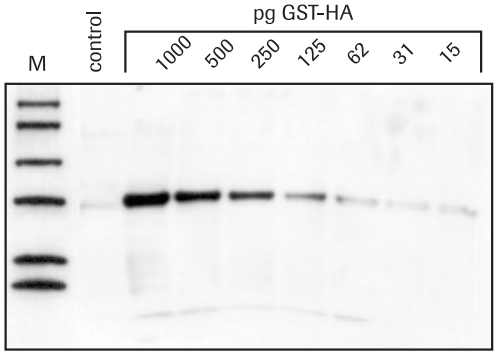

Fig. 1: Immunoblot of a HA-tagged GST-fusionprotein (GST-HA) serially diluted in an untransfected eucaryotic cell extract (10 µg total protein per lane) and indirectly detected with Anti-Rat-Ig-Biotin* (20 ng/ml) and Streptavidin-POD* (10 mU/ml) using BM Chemiluminescence Blotting substrate (POD)*. The concentration of Anti-HA High Affinity was 100 ng/ml. The control lane is an untransfected eucaryotic cell extract (10 µg total protein total protein).
M: MULTI-TAG-MARKER I.

Immunoprecipitation

Additional reagents required

- Lysis buffer (*e.g.*, RIPA buffer: 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF*, 1 mg/ml Leupeptin*, 5 mg/ml Aprotinine, 1% Nonidet 1) P40*, 0.5% sodium deoxycholate, 0.1% sodium dodecylsulfate)
- Protein G Agarose*

Protocol

Please refer to the following table.

Step	Action
1	Lyse cells with an appropriate Lysis for 30 min on ice.
2	• Centrifuge for 5 min in a microfuge at maximum speed. • Transfer supernatant to a new reaction vial.
3	Presorbe supernatant with Protein G Agarose* for 1 – 3 h (or overnight) at +2 to +8°C.
4	• Centrifuge for 1 min in a microfuge at maximum speed. • Transfer supernatant to a new reaction vial.
5	• Add Anti-HA High Affinity to the supernatant to a final concentration of 0.5–5 µg/ml. • Incubate 1 – 3 h (or over night) at +2 to +8°C.
6	Collect the immune-complex by the addition of Protein G Agarose* and further incubation for 1 – 3 h at +2 to +8°C.
7	Wash beads thoroughly with lysis buffer before further analysis

ELISA

IF you use Anti-HA (3F10) as	Then...
capture antibody	use a 1 – 5 µg/ml IgG in 50 mM sodium carbonate buffer, pH 9.6 for coating. Incubate 100 µl/well for 2 h at +15 to +25°C or over night at +2 to +8°C.
detection antibody	incubation with an antibody concentration of 100 ng/ml in TBS containing 1% BSA or Blocking Reagent at +15 to +25°C for 1 h is recommended.

References

1 Wilson, I.A. et al. (1984) *Cell* **37,** 767-778.
2 Field, J. et al. (1988) *Mol. Cell. Biol.* **8,** 2159-2165.
3 Kolodziej, P.A. and Young, *R.A. Methods Enzymol.* **194,** 508-511.
4 von Zastrow, M. and Kobilka, B.K. (1992) *J. Biol. Chem.* **267,** 3530-3538.
5 Kolodziej, P.A. et al. (1990) *Mol. Cell Biol.* **10,** 1915-1920.
6 Wadzinski, B.E. et al. (1992) *J. Biol. Chem.* **267,** 16883-16888.
7 Pines, J. and Hunter, T. (1991) *J. Cell Biology* **115,**1.
8 Canfield, V.A. and Levenson, R. (1993) *Biochemistry* **3,** 13782.
9 Antebi, A and Fink, G.R. (1992) *Mol. Biol. of the Cell* **3,** 633.
10 Sherwood, P.W et al. (1993) *Mol. Cell. Biol.* **13,** 28.
11 Shen, W.C. et al. (1993) *J. Biol. Chem.* **268,** 19436.
12 Herscovics, A. et al. (1994) *Biol. Chem.* **269,** 9864.
13 Manolson, M.F. et al. (1994) *J. Biol. Chem.* **269,** 14064.
14 Berkower, C. et al. (1994) *Molec. Biol. of the Cell* **5,** 1185.
15 Templeton, D.J. (1992) *Mol. Cell. Biol.* **12,** 435.
16 Zhou, O. et al. (1992) *Genes & Development* **6,** 1946.
17 Emrich, T. et al. (1994) *Biochem. Biophys. Res. Com.* **197,** 214-220.
18 Chen, Y.-T. (1993) *Proc. Natl. Acad. Sci.* **90,** 6508-6512.
19 Emrich, T. et al. (1996) 6th European Biosymposium, Montpellier, France.
20 Harlow, E. & Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N.Y.

Related products

Product	Pack size	Cat. No.
HA Peptide	5 mg	11 666 975 001
Anti-HA-Biotin, High Affinity (3F10)	50 µg	12 158 167 001
Anti-HA-Fluorescein, High Affinity (3F10)	25 µg	11 988 506 001
Anti-HA-Peroxidase, High Affinity (3F10)	25 U (25 µg)	12 013 819 001
Anti-HA Affinity Matrix (3F10)	1.0 ml	11 815 016 001
Anti-HA (12CA5)	200 µg	11 583 816 001
Anti-HA-Biotin (12CA5)	100 µg (500 µl)	11 666 851 001
Anti-HA-Fluorescein (12CA5)	100 µg (500 µl)	11 666 878 001
Anti-HA-Rhodamine (12CA5)	100 µg (500 µl)	11 666 959 001
Anti-c-myc	200 µg	11 667 149 001
Anti-c-myc-Peroxidase	500 µg	11 814 150 001
c-myc peptide	5 mg	11 667 246 001
Anti-His6	100 µg	11 922 416 001
Anti-His6-Peroxidase	50 U	11 965 085 001
Anti-VSV-G	200 µg	11 667 351 001
Streptavidin-POD	500 U	11 089 153 001
Anti-Biotin-POD, Fab fragments	150 U	11 426 311 001
PVDF Western Blotting Membranes	1 roll, 30 cm × 3 m	03 010 040 001
Lumi-Light Western Blotting Substrate	400 ml (4000 cm^2 membrane)	12 015 200 001
Lumi-LightPlus Western Blotting Substrate	100 ml (1000 cm^2 membrane)	12 015 196 001
BM Chemiluminescence Blotting Substrate (POD)	1000 cm^2 4000 cm^2	11 500 708 001 11 500 694 001
Immunoprecipitation Kit (Protein A)	20 reactions	11 719 394 001
Immunoprecipitation Kit (Protein G)	20 reactions	11 719 386 001
Protein A	2 ml	11 719 408 001
Protein G	2 ml	11 719 416 001
Blocking Reagent	50 g	11 096 176 001
Western Blocking Reagent, Solution	100 ml (10 blots, 100 cm^2) 6 × 100 ml (60 blots, 100 cm^2)	11 921 673 001 11 921 681 001
FuGENE® 6 Transfection Reagent	0.4 ml 1 ml Multi-pack 5 × 1 ml	11 815 091 001 11 814 443 001 11 988 387 001
FuGENE® HD Transfection Reagent	0.4 ml, (up to 120 transfections), 1.0 ml (up to 300 transfections), 5 × 1 ml (up to 1,500 transfections),	04 709 691 001 04 709 705 001 04 709 713 001

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